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Application of qPCR assays for diagnosing causes of viral mink diarrhea. Preliminary results.

Christina Marie Hartby, Lise Kirstine Kvisgaard, Lars Erik Larsen, Mariann Chriél, Charlotte Kristiane Hjulsager.

National Veterinary Institute, Technical University of Denmark, 1870 Frederiksberg, Denmark.

### Abstract

Gastrointestinal (GI) disorders is the main cause for submitting mink (*Neovison vison*) carcasses for post-mortem examination at the National Veterinary Institute in Denmark and has been described as the predominant cause of disease and mortality in the Danish mink production (Rattenborg et al. 1999). Diarrhea in mink can be caused by infectious agents (virus, bacteria and parasites) and food-related/multifactorial conditions. Known enteric viral infections are mink enteritis virus (MEV) and mink astrovirus. Coronaviruses and caliciviruses have also been implicated as potential causes or contributors to diarrhea in mink. Rotavirus is poorly described in mink, but has previously been demonstrated in feces from mink pups with and without clinical signs (Jorgensen et al. 1996). The pathogenicity of these viruses could be related to viral load, virulence and the age of the mink. Therefore, there is a need for a quantitative diagnostic approach. We have developed new or adapted previously published real-time PCR/RT-PCR assays for MEV, astrovirus, rota- and coronavirus diagnostics.

The technical test validation was initially carried out on archived diarrhea samples from diagnosed positive animals and on normal and diarrhea samples from a case-control study. In order to further validate the applicability of the assays, a testing scheme for normal and affected farms was set up and initiated in June 2015. This protocol will allow optimization of test characteristics (sensitivity, specificity and predictive value) and assessment of the validity of using pooled samples in order to reduce test costs.

### Dansk resumé

Gastrointestinale (GI) lidelser er en dominerende årsag til at indsende mink (*Neovison vison*) kadavere til post-mortem undersøgelser på Veterinærinstituttet i Danmark, og er beskrevet som den overvejende årsag til sygdom og dødsfald i den danske minkproduktion (Rattenborg et al. 1999). Diarré i mink kan skyldes både infektiøse agens, såsom virus, bakterier og parasitter og foderrelaterede/multifaktorielle tilstande. Kendte enteriske virusinfektioner er mink enteritis virus (MEV) og mink astrovirus. Coronavirus og calicivirus har også været sat i forbindelse med diarré

som overvejende eller medvirkende årsag hos mink. Rotavirus er kun mangelfuldt beskrevet hos mink, men tilstedeværelsen har tidligere været demonstreret i fæces fra minkhvalpe både med og uden kliniske tegn (Jorgensen et al. 1996). Patogeneciteten af disse vira kan være relateret til dosis af virus, virulens og minkens alder. Derfor er der et behov for en kvantitativ diagnostisk tilgang. Til dette formål har vi udviklet nye eller tilpasset publicerede real-time PCR eller RT-PCR assays for MEV, astrovirus, rota- og coronavirus diagnostik.

Indledningsvise undersøgelser blev foretaget ved test af arkiverede diarréprøver fra diagnosticerede inficerede dyr og af normale og diarré prøver fra et case-control studie. For at validere anvendeligheden af disse assays yderligere, er en ny prøveindsamling påbegyndt i juni 2015, som skal tilvejebringe prøver fra normale og diarre problembesætninger. Testresultater fra prøverne vil muliggøre optimering af test karakteristika (sensitivitet, specificitet og prædiktiv værdi) samt validere validiteten ved brug af poolede prøver for at mindske testomkostninger.

## Materials and methods

### *Study design*

Feces samples are being collected from June until the pelting season begins in the middle of October in three categories: 1) Samples from mink submitted for diagnosis at the National Veterinary Institute at the Technical University of Denmark; 2) Twelve farmers submit feces from five young healthy animals every second week– these serve as the baseline; 3) Veterinarians submit samples from five healthy and five diarrheic animals, whenever they visit a farm where diarrhea is present – these make up the case-control samples.

### *Real-time PCR*

Nucleic acids were purified with QIA-symphony DSP Virus/Pathogen Mini Kit (QIAGEN) from feces samples diluted 10-fold in PBS buffer. The presence of rotavirus and astrovirus was tested with adapted real-time RT-PCR assays (Pang et al. 2004; Ullman et al. 2004). The presence of MEV was tested by an in-house developed real-time PCR assay.

## Results

Currently MEV has not been detected in any of the samples tested.

Astrovirus has been detected in most of the submissions, without correlation to reported diarrhea or not. The quantitative level of virus varies, but there seems to be a correlation between a high level of virus and the occurrence of “sticky kits”.

Rotavirus has been detected in a single submission. Typing is ongoing.

## Discussion and conclusion

The knowledge on viral infectious causes of diarrhea in mink is very limited. By the present study we aim to describe the occurrence of viruses in mink with and without diarrhea to delimitate the causative agents. By collection of samples from June to October, we hope to reveal any seasonal patterns of the level of viruses in healthy animals and the viral load patterns in different age groups. The preliminary results for astrovirus highlights the need for a quantitative approach in combination with an assessment of multiple viral agents to increase diagnostic accuracy.

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